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ON EPINEPHRIN AND ITS COMPOUNDS, WITH ESPECIAL REFERENCE TO EPINEPHRIN HYDRATE.[1]

(An investigation now being carried on under a grant from the Carnegie Institution.)

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The first important contribution to our knowledge of the chemical composition of the suprarenal gland or capsule was furnished by Vulpian,[2] who, in 1856, observed that the fluid expressed from the interior part of the capsules of many different animals behaves in a striking manner toward ferric chloride and also toward solutions of iodine and other oxidizing agents, and that with no other fluid of the body can similar reactions be obtained.

This juice of the medullary substance, which darkens on exposure to the air, is the *atra bilis* of the older anatomists. Vulpian found that it gave a fine green color with salts of iron, a pink or rose-red with iodine, water and ammonia, but he and the men of his time were unable to isolate the substance. The immediate cause of the renewed interest of chemists in this field was the discovery of Schäfer and Oliver[3] and of Cybulski and Szymonowicz[4] in 1894 and

[1] The material used in this paper is drawn from two articles now in press. One of these articles is to be found in the "Contributions to Medical Research," dedicated to V. C. Vaughan, the other in the *Berichte d. deutsch. chem. Gesellschaft*, Berlin. In the first-named article I have discussed the question as to how far epinephrin fulfills the requirements of a specific product of internal secretion, and have also reviewed the evidence which proves that the suprarenal capsules are functional structures and essential to the continuance of life.

[2] *Compt. rendus*, Acad. d. Sc., Paris, Vol. 93, pp. 663–665.

[3] *Jour. of Physiology*, Vol. 16 (1894), Vol. 18 (1895).

[4] *Archiv f. die gesammte Physiol.*, Vol. 64, p. 149.

1895, that a very minute amount of an aqueous extract of the medulla of the gland will raise the arterial pressure to an extraordinary degree. Interest in the subject of internal secretion was at this time at its height. Pellacani and his pupils had studied the effects of the injections of extracts of the gland in 1879; others followed, but Schäfer and Oliver were the first to test the effects of extracts on the arterial pressure. As early as 1885, Krukenberg[1] had declared that the substance which gives the green color with ferric chloride is not the chromogenic substance of Vulpian, but more likely pyrocatechin accompanying the chromogen. From this time on, the opinion that pyrocatechin is present in the gland gained a firm hold. Brunner[2] found that an alcoholic extract of the gland can be made to give nearly all of the reactions of pyrocatechin; thus, it gives the well-known green color with ferric chloride, passing into a fine red on the addition of ammonium tartrate and a few drops of an alkali; it redúces silver nitrate at room temperature and Fehling's solution on boiling. The addition of an alkali soon causes it to take on a dark-brown color; lead acetate gives a precipitate; sodium nitroprusside and very dilute ammonia give a wine-red color. All of the above reactions being given also by pyrocatechin, Brunner concluded that Krukenberg was right in his belief that pyrocatechin is present in the suprarenal gland.

Again, in 1896, after it was known that the gland contained a blood-pressure-raising constituent, Mühlmann[3] returned to the pyrocatechin theory, but modified it so far as to state that pyrocatechin is not present *as such* in the gland, but in a form of combination with the active principle, from which it may be split off by boiling with dilute hydrochloric acid.

It will be seen that at this time there was a great diversity of opinion as to the chemical character of the blood-pressure-raising constituent. No one had proved the existence of pyrocatechin in the gland, either as such or in combination with the active principle, by such irrefutable evidence as precipitation with lead acetate and analysis of the lead compound thus obtained, and no one had even

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[1] *Arch. f. path. Anat.*, Bd. CI (1885), pp. 542–591.
[2] *Schweiz. Wochenschr., f. Pharmacie*, Bd. XXX, 1892, pp. 121–123.
[3] *Deutsche med. Wochenschr*, 1896, No. 26, pp. 409–411.

roughly isolated the blood-pressure-raising constituent in the form of a definite chemical compound.

In 1895 B. Moore,[1] working in Schäfer's laboratory, came to the conclusion that Vulpian's chromogen and the blood-pressure-raising constituent are identical. He based this opinion on the fact that chemical operations which destroy the color reactions of the chromogen also deprive the blood-pressure-raising constituent of its power to raise the arterial pressure. That the solubilities of the active principle are the same as those of the chromogen appeared also to support this opinion.

Fraenkel,[2] working with residues obtained with the help of alcohol and acetone as solvents, also concluded that the blood-pressure-raising constituent and Vulpian's chromogen are one and the same substance. His residues did not contain pyrocatechin, and he concluded from their behavior toward ferric chloride and other oxidizing agents that the essential principle of these residues is a nitrogenous derivative of the *ortho*dihydroxy-benzene series.

In 1897 Abel and Crawford[3] showed that the active principle may be precipitated from *aqueous* extracts of the gland by treatment with benzoyl chloride and sodium hydrate. Fraenkel[4] had, the year before, shown that a syrupy substance is thrown out when benzoyl chloride is shaken up with a *pyridin* solution of an extract of the gland, but he did not attempt to decompose it in order to ascertain whether it was in reality a benzoyl derivative of the blood-pressure-raising constituent. Abel and Crawford, however, were unaware of the fact that Fraenkel had made trial of benzoyl chloride in a pyridin solution, until their own work was nearly completed. These writers decomposed their benzoyl product with hot dilute sulphuric acid and obtained the active principle in the form of a sulphate of a tarry consistency, which possessed great physiological activity, gave the color reactions of Vulpian, reduced silver nitrate, and possessed the other specific qualities of suprarenal extracts. They did not succeed in splitting off pyrocatechin from their active principle by boiling with dilute hydrochloric acid. They also stated that their sul-

[1] *Jour. Physiol.*, XVII, 1895; *Proc. Physiol. Soc., London*, p. xiv; *ibid.*, Vol. XXI, 1897.

[2] *Wiener med. Blätter*, 1896, Nos. 14–16.

[3] *The Johns Hopkins Hospital Bulletin*, July, 1897, No. 76.

[4] *Loc. cit.*

phate did not reduce Fehling's solution. As will be seen later, their failure to obtain a reduction with Fehling's solution was due to the fact that they did not boil the solution for a sufficient length of time. While opposed to Brunner on this point, they were in full agreement with v. Fürth,[1] Metzger[2] and Moore,[3] all of whom, also, at this time took the ground that extracts of the suprarenal gland do not reduce Fehling's solution. This point is of minor importance only, as it concerns but one among the reactions of the blood-pressure-raising principle. We shall refer to it again in a later section of this paper.

In two subsequent papers[4] the following statements, among others, were made by the writer in reference to the active principle which he now named epinephrin.

(1) Epinephrin is a basic substance. It is thrown out of its solution in acids by ammonia in the form of an amorphous flocculent compound, which rapidly loses its power to raise the arterial pressure.

(2) Both the free base and its salts give reactions with a considerable number of alkaloidal reagents.

(3) Both the free base and its salts give the color reactions of Vulpian, reduce silver nitrate, and in all other ways agree with what is known in respect to Vulpian's chromogen.

(4) The formula $C_{17}H_{15}NO_4$ was adopted as expressing the elementary composition of epinephrin, this formula being based on the analyses of a considerable series of salts and derivatives, all of which were obtained by saponification of the original benzoyl compound by means of very dilute sulphuric acid in an autoclave.

(5) After saponifying this benzoyl product in the manner just described, the liberated base was first precipitated as a picrate, and from this picrate other salts such as the bisulphate were formed by simple transposition in the proper medium. The salts thus prepared possessed a high degree of physiological activity. Singularly enough, however, on precipitating the free base from these active salts it was found to have little power to raise the blood-pressure.

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[1] *Zeitschr. f. physiol. Chem.*, XXIV, p. 142.

[2] Zur Kenntniss der wirksamen Substanzen der Nebenniere, Diss. Würzburg, 1897.

[3] *Jour. Physiol.*, Vol. XXI, 1897.

[4] Abel: *Johns Hopkins Bulletin*, September–October, 1898, Nos. 90–91; *Zeitschrift f. physiol. Chem.*, Bd. XXVIII, pages 318–362.

(6) The addition of an alkali, as sodium hydrate, to the free base or one of its salts produces two substances, one of which is a basic body of a peculiarly intense and nauseating odor, reminding one of a mixture of coniin and piperidin; the other, a dark pigment of an acid character. This latter decomposition product was called epinephrinic acid.

(7) On smelting epinephrin with powdered alkalies, a small quantity was obtained of a substance that corresponded in its reactions to skatol. Dry distillation with zinc dust in a current of dry hydrogen yielded amines, benzaldehyde, and pyrrol. On heating epinephrin bisulphate in a sealed tube with 25 per cent. hydrochloric acid to 150° C., a small amount of an ether-soluble substance was obtained which took on a green color on the addition of ferric chloride. No attempt was, however, made to identify this body with pyrocatechin or pyrocatechinic acid. An acid with a melting point of 120° C. and all the properties of benzoic acid was also obtained by this treatment. In consequence of these reactions, epinephrin was declared to belong in a general way to the class of pyrrol derivatives.

The Work of von Fürth.—Shortly after the appearance of the paper by Abel and Crawford, v. Fürth entered the field. On the basis of a series of analyses of an impure acetyl product prepared directly from crude extracts of the gland, this writer[1] assumed the blood-pressure-raising constituent to be either tetrahydrodioxypyridin, $C_5H_9NO_2$, or dihydrodioxypyridin, $C_5H_7NO_2$. No more need be said in reference to this claim since v. Fürth[2] has himself lately abandoned this theory. He has, however, made some contributions of value, notably the isolation of the blood-pressure-raising principle in the form of a physiologically active, though impure, iron compound. This compound is, however, so difficult to purify that its analysis throws but little light on the true elementary composition of our substance. V. Fürth furnished no analyses in connection with his earlier work on this iron compound. In those furnished later, he assumes by comparison with the commercial preparation, adrenalin, that those fractions of his iron compound which show the highest percentage of carbon most nearly represent

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[1] *Zeitschr. f. physiol. Chem.*, Bd. XXIV, page 142; XXVI, page 15; XXIX, page 105.

Beiträge zur chem., Physiol. u. Pathol., Bd. I, page 243.

the true composition of the blood-pressure-raising constituent which he had called suprarenin. No rational formula was deducible from the analyses, but the expression $C_{8\cdot5}H_{12\cdot2}NO_x$ was adopted as representing the average composition of the fractions analyzed.

It will be remembered that the writer had laid stress on the fact that the blood-pressure-raising principle as isolated by him is characterized by alkaloidal properties and is basic in character as shown by its ability to unite with acids and by the fact that it is precipitated by ammonia. It was claimed by v. Fürth that his suprarenin, as he now called the blood-pressure-raising constituent, exhibits none of these characteristics, and he did not hesitate to say that epinephrin itself has no connection with the active principle, but is, in fact, a very different substance slightly contaminated with the true blood-pressure-raising constituent.

It was, however, an easy matter to show that differences in method are responsible for the observed differences in the properties of epinephrin and suprarenin. The benzoyl and acetyl derivatives of epinephrin were prepared from the iron compound of v. Fürth and were saponified in the autoclave. The resulting solutions were found to yield flocculent epinephrin on the addition of ammonia. In a word, it was found that treatment in the autoclave of any form of the active principle causes it to assume alkaloidal properties and to become precipitable by ammonia. It was furthermore shown that epinephrin as obtained by saponification of its benzoyl derivative is convertible into an iron compound which is qualitatively indistinguishable from that prepared by v. Fürth's method, though containing a benzoyl radical which was not removed in the saponification of the original benzoyl compound.

To summarize briefly, it was shown,[1] in reply to the criticisms of v. Fürth, that:

(1) It is an inherent property of the active principle of the suprarenal gland, prepared by whatever method, to fall out in the form of a flocculent, physiologically inactive precipitate on the addition of ammonia, *after previous treatment in the autoclave.*

(2) It was also shown that when suprarenin or any other form of the active principle was subjected to hydrolytic treatment in the

[1] "Further Observations on Epinephrin," *Johns Hopkins Hospital Bulletin*, Vol. XII, March, 1901.

autoclave, it not only acquired the above-named property, but also assumed alkaloidal characteristics.

(3) It was also proved that epinephrin gives an iron compound which is qualitatively indistinguishable from v. Fürth's iron compound, and that the iron compound made from *free epinephrin* is identical with that obtained from *epinephrin bisulphate*, whereas v. Fürth had maintained that only the *filtrate* from free epinephrin could yield an iron compound.

(4) In a subsequent paper[1] it was clearly proved that adrenalin, which, like suprarenin, is devoid of alkaloidal properties, could be made to assume all the alkaloidal and other characteristics of epinephrin on mere solutions in mineral acids.

(5) It was further shown that on benzoating or acetylating the iron compound of v. Fürth, and on decomposing these derivatives, a series of compounds could be prepared which are qualitatively indistinguishable from the earlier epinephrin compounds.

(6) In a later paper[2] it was described how the iron compound of v. Fürth was converted into the acetyl derivative, how this was then saponified in the autoclave so that a picrate and from this in turn a bisulphate could be prepared, and how this bisulphate was again converted into the acetyl compound. On analysis this acetyl derivative gave data that agreed well with those required for the triacetyl derivative of an oxy-epinephrin, $C_{10}H_{11}NO_4$.

(7) In the paper just alluded to it was also shown that my early empirical formula for epinephrin, $C_{17}H_{15}NO_4$, was too large by one benzoyl group, $CO \cdot C_6H_5$, and that this was a consequence of the singular tenacity with which this lone radical defied all but destructive methods for its removal from the original benzoyl compound. On heating epinephrin with strong sulphuric acid, benzoic acid is split off from it. Neither suprarenin nor adrenalin yield this acid on similar treatment. Elimination of this retained radical from my former series of compounds and substitution of the displaced hydrogen atom led to the formula $C_{10}H_{11}NO_3$ as an adequate empirical expression for epinephrin with alkaloidal properties.

Without going further into the details of the controversy, it will

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[1] "On a Simple Method of Preparing Epinephrin and Its Compounds," *Johns Hopkins Hospital Bulletin*, Vol. XIII, 1902.

[2] *Johns Hopkins Hospital Bulletin*, November, 1901.

be apparent that my earlier statements in regard to the basic and alkaloidal properties of epinephrin were entirely correct, and that such of these properties as are not possessed by suprarenin can be conferred upon it by appropriate treatment.

It will also be apparent that while much evidence has been brought forward in my whole series of papers in support of the statement that alkaloidal epinephrin is a chemical individual whose composition is represented by the formula $C_{10}H_{11}NO_3$, and while it has been clearly proved that suprarenin may easily be converted into epinephrin, nothing is definitely known in regard to the chemical composition of suprarenin.

ON ADRENALIN.

While the writer was engaged in refuting the statements of v. Fürth, a crystalline preparation of the active principle was made by Takamine.[1]

His method, a very simple one, is based on his observation that *ammonia precipitates the active principle directly from a sufficiently concentrated aqueous extract of the gland.*

Although I had demonstrated that epinephrin is a basic substance, and although I had repeatedly shown that it can be precipitated as a flocculent substance by ammonia, the fact that it could be precipitated in a physiologically active and crystalline condition directly from gland extracts had escaped me because I used either an insufficient quantity of ammonia or too dilute a solution of the active principle. In his first paper, Takamine gave no description of his method and no analytic data as to the elementary composition of his substance, but only a brief list of well-known reactions which his substance gave in common with the preparations of others. He named his preparation adrenalin.

Shortly after the appearance of this paper, Aldrich[2] also prepared the free base in the active and crystalline condition and likewise by the use of ammonia or sodium carbonate as a precipitant. His method differs from that of Takamine only in certain unessential details, such as the use of lead acetate for the removal of inert substances, as originally advised by Holm and later by v. Fürth.

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[1] First paper, *Ther. Gazette*, Vol. XXV, p. 221; second paper, AM. JOUR. PHARM., Vol. LXXIII, p. 523.

[2] *Am. Jour. Physiol.*, Vol. V, p. 457.

Aldrich's substance is, of course, quantitatively and qualitatively identical with the adrenalin of Takamine, and he has, therefore, used this name. He analyzed his own compound and also purified and analyzed the adrenalin of Takamine with the result that "the simplest body obtainable is represented by the formula $C_9H_{13}NO_3$."

Finally Takamine[1] described his own method already outlined, and also published analyses of his own adrenalin, which differ very materially from those obtained by Aldrich. He asserted, indeed, that his formula differs from that of Aldrich by CH_2 and adopted the expression $C_{10}H_{15}NO_3$ as the "probable empirical formula" of adrenalin. We have, then, an inexplicable lack of agreement in the analyses of what is evidently one and the same substance, as judged by the descriptions given by these writers.

Neither of these writers has placed the theoretical values required by his formula side by side with those actually obtained in the analyses. When this is done, as in the following table, it is seen that neither formula can be accepted on the evidence which has thus far been furnished.

Aldrich's Adrenalin Found.	Found, for Takamine's Adrenalin as Purified and Analyzed by Aldrich.	Theoretical for $C_9H_{13}NO_3$, as Proposed by Aldrich.
C = 57·89	C = 58·03	C = 59·02
H = 7·33	H = 7·20	H = 7·10
N = 7·50	N = 7·66	N = 7·65
O = 27·27	O = 27·11	O = 26·23
99·99	100·00	100·00

Adrenalin as prepared and analyzed by Takamine:

Found.	Required for $C_{10}H_{15}NO_3$, as Proposed by Takamine.
C = 59·39	C = 60·91
H = 7·84	H = 7·61
N = 7·88	N = 7·11
O = 24·89	O = 24·37
100·00	100·00

The very great deficiency of carbon in the analyses of both Aldrich and Takamine, as compared with the theoretical requirements for this element, is alone sufficient to condemn the proposed formulæ. The case, moreover, is such that no rational formula

[1] See his second paper as already cited.

whatsoever is deducible from the above analytic data, a sure indication that adrenalin, as hitherto isolated, is a mixture and not a chemical individual. The fact that adrenalin is so readily convertible into the alkaloidal modification suggests at once that a simple relationship must exist between these two modifications of the blood-pressure-raising principle, quite aside from the question as to whether an atomic rearrangement has occurred in the process of conversion.

ON THE TRUE ELEMENTARY COMPOSITION OF THE ABOVE-DESCRIBED CRYSTALLINE, NON-ALKALOIDAL FORM OF EPINEPHRIN.

Since neither Aldrich nor Takamine has succeeded in isolating adrenalin as a pure substance, and also because it is necessary to establish the relationship existing between epinephrin and this substance, I have undertaken to purify and to analyze it. As prepared by the zinc-ammonia process described by me in an earlier paper,[1] and washed *entirely free* of ammonia with water, alcohol and ether and then dried in vacuo over sulphuric acid, adrenalin is found to be stable as long as it is kept perfectly dry. As made by me according to the process just named, redissolved in dilute hydrochloric acid and again precipitated with ammonia, its composition was found to be:

C = 57·39 to 57·60
H = 6·29 to 6·77
N = 7·38 (Kjeldahl-Gunning).

After nine precipitations with ammonia or sodium carbonate its composition changed to:

I.	II.	Required for $C_{10}H_{13}NO_3 . \frac{1}{2}H_2O$.
C = 58·61	58·67	C = 58·82
H = 6·84	6·77	H = 6·86
N = 7·08 (Kjeldahl-Gunning[2])		N = 6·86
O = 27·47		O = 27·46
100·00		100·00

[1] *The Johns Hopkins Hospital Bulletin*, February-March, 1902, Vol. XIII.

[2] In applying this method it is necessary to continue the digestion with the concentrated sulphuric acid for about four hours in order to effect the complete decomposition of the substance. I was for a time of the opinion that the Kjeldahl-Gunning method does not liberate all of the nitrogen as ammonia (see *Amer. Jour. of Physiology*, Vol. VIII, 1903, No. 5), but it was afterwards found that the control analysis by the method of Dumas was at fault.

Analytical data:

0·2508 gramme substance gave 0·5390 gramme CO_2, and 0·1545 gramme H_2O; that is, C = 58·61 per cent. and H = 6·84 per cent.

0·3633 gramme substance required 7·59 c.c. of sulphuric acid, 1 c.c. of which corresponded to 0·003392 gramme N, for the neutralization of the obtained ammonia (Kjeldahl-Gunning); therefore, N = 7·08 per cent.

0·2025 gramme substance gave 0·4356 gramme CO_2 and 0·1234 gramme H_2O; that is, C = 58·67 per cent. and H = 6·77 per cent.

The above analytical data have been fully substantiated by my latest work. A much simpler and cheaper method than the zinc-ammonia process, one which gives an extraordinarily large yield of our substance in a pure form is the following. For convenience I shall speak of it as the trichloracetic acid method. It may best be described by taking an actual example from my note-books:

For example, 11·13 kilogrammes of beeves' glands, weighed after being freed of adherent fat and tissue, were finely minced and the mass divided among a number of 2½-litre flasks, so that they were about half filled. Five litres of absolute alcohol, containing 175 grammes of trichloracetic acid, was now equally divided among the flasks, added in very small quantities at a time with vigorous shaking.

Great care must be taken in this operation in order that the acidulated alcohol may well penetrate the tissues and mix with their water instead of merely coagulating and hardening the individual particles. This mixture is allowed to stand over night and then subjected to filtration under pressure. To the almost colorless filtrate more absolute alcohol may be added until no further precipitation occurs. Later work has shown that this is an unnecessary step in the process. I now proceed directly to the evaporation under diminished pressure of the first alcoholic filtrate. The 5 or 6 litres thus obtained are concentrated to a volume of 380 c.c. This small volume contains a flocculent precipitate which is removed by filtration under pressure. To the clear filtrate it is only necessary to add ammonia of specific gravity 0·944, stirring gradually, when a veritable rain of crystals is seen falling to the bottom of the beaker.

As soon as the solution smells permanently of ammonia, the precipitation will be found to be complete and no further crystals are obtained.

The crystalline precipitate is immediately filtered and subjected to a *prolonged* washing with water and to a shorter washing with absolute alcohol and ether, and it is then dried over sulphuric acid. When dry, the material, which is already almost snowy-white, was found to weigh 23·79 grammes.

Owing to the very great solvent power of trichloracetic acid, even in absolute alcohol, for earthy phosphates and other salts, the precipitate just described is considerably contaminated with mineral constituents (phosphates), even to the extent of 10 or 12 per cent.; but even so, the material seems pure enough in other respects for all local therapeutic applications in the strength of solution usually employed. As regards these mineral constituents and other possible impurities contained in the crystalline precipitate, it will presently be shown how easily these may be removed.

But in spite of the large yield of the active principle obtained by this process, it will be found to be of great advantage to repeat the extractions one or more times with the following modification:

To the once-exhausted glands, 5 or 6 litres, not of absolute, but of 60 or 70 per cent. alcohol, containing 30 or 40 grammes of trichloracetic acid, are added and the whole process of filtration, evaporation and precipitation proceeds as before. During the evaporation of these later extractions care must be taken that they maintain an acid reaction, as otherwise great injury will be done to the active principle, and one runs the risk of obtaining a final product consisting largely of phosphates, the active principle remaining in solution in a partially oxidized or otherwise altered form.

From the weight of glands (11·13 kilogrammes) used in the above instance I obtained from the second extract 8·57 grammes; from the third extract 3 grammes. It will thus be seen that the sum total of crystalline, though somewhat impure, material obtained from 11·13 kilogrammes of trimmed glands amounted to 35·36 grammes. When we take into consideration that the glandular material is not yet completely exhausted, that with better appliances for filtration a still better yield could be obtained, it is apparent that the amount of the active principle contained in beeves' glands has been hitherto underrated, and that it may safely be assumed as constituting at least 0·3 per cent. of the moist gland. It need only be added that the cheaper methyl-alcohol will no doubt serve equally well for extraction. I have, however, as yet found no other acid to equal

trichloracetic acid in penetrating the tissue, dissolving out the active principle and producing so light-colored a product.

METHOD OF PURIFYING THE ABOVE PRODUCT.

Twenty-three grammes of the crystalline material obtained from first extractions was stirred up in 80 c.c. of water containing 6 grammes of oxalic acid, and then 800 c.c. of absolute alcohol was added in small quantities, accompanied by vigorous stirring. Ether was then added until the total volume was nearly 1 litre. The flask is then set aside for a day or more, when the alcohol-ether fluid can be poured off from the abundant sticky precipitate. Relatively little of the substance is found in this alcohol-ether fluid in the form of an oxalate, and may easily be recovered from it only slightly contaminated with ash by simple precipitation with ammonia.

The gummy precipitate, which contains almost all of our substance, is dissolved as far as possible in about 50 c.c. of water containing 12 or more grammes of trichloracetic acid. It is then precipitated with absolute alcohol, added in small quantities at a time until 800 c.c. has been added. Approximately 150 c.c. of ether is then added and the whole set aside until the supernatant liquid is clear. The mineral constituents (alkaline earths) will then be found to have been thrown down in the form of a white precipitate, while the active principle, together with some water-soluble mineral constituents, is contained in the alcohol-ether fluid. From this fluid it is now obtained by precipitation by ammonia. After being very thoroughly washed with water, alcohol and ether it is found to be entirely ash-free.

For purposes of analysis it is only necessary to recrystallize it by precipitation with ammonia from a solution in aqueous hydrochloric or trichloracetic acid. For example, a portion of the white amorphous precipitate obtained by ammonia from the alcohol-ether mixture just referred to was dissolved in dilute oxalic acid, precipitated by ammonia; again dissolved in dilute trichloracetic acid and thoroughly washed with water that has been freed of carbonic acid, then with alcohol and ether and dried in vacuo over sulphuric acid. As thus prepared, especially when the precipitation with ammonia is not effected too rapidly, the substance forms nodular aggregates of well-formed microscopic prisms terminated sharply by pyramidal planes.

Its composition was found to be:

I.	II.
C = 58·72	C = 58·73
H = 6·87	H = 6·84
N = 7·12	N = 7·15

Analytical data:

I. 0·2200 gramme substance yielded 0·4737 gramme CO_2 and 0·1360 gramme H_2O; therefore, C = 58·72 and H = 6·87.

0·3557 gramme substance gave 21·8 c.c. N collected over 50 per cent. KOH at t = 21° C. and barometer = 760 mm. N = 7·12.

II. 0·2438 gramme substance yielded 0·5250 gramme CO_2 and 0·1500 gramme H_2O; therefore, C = 58·73 and H = 6·84.

0·3215 gramme substance gave 19·8 c.c. N, collected over 50 per cent. KOH at 21° C. and 760 mm. Hg. N = 7·15.

Identical analytical results have been obtained by purifying commercial preparations known as suprarenalin (Armour & Co.) and adrenalin (Parke, Davis & Co.).

At the time these analyses were made I had not yet employed oxalic and trichloracetic acids conjointly, as above described. The suprarenalin of Armour & Co. was dissolved in absolute alcohol containing trichloracetic acid, filtered and precipitated by ammonia. It was then dissolved in as little dilute oxalic acid as possible, and ammonium picrate and picric acid were added. This solution was allowed to stand, and then filtered from a small amount of a dark, sticky picrate. The active principle was then precipitated by ammonia and thoroughly washed with water, alcohol and ether.

It was then dissolved in dilute hydrochloric acid and crystallized with the slow addition of ammonia.

It was now found to have the composition

C = 58·58 H = 6·80 N = 7·09 (Dumas).

Nearly a year ago I purchased large amounts of adrenalin and subjected it to processes of fractional precipitation. The extreme variations of numerous fractions in respect to C, H, and N, ran from

C = 56·53 to 58·89
H = 4·77 to 7·19
N = 7·59 to 10·65 (Dumas).

It would, however, be an easy matter to obtain results exactly like those above cited from adrenalin, if my present methods of purification were applied to this substance. Even simple reprecipitation (four times) with ammonia from its solution in acetic or

hydrochloric acid often gave a crystalline material for the most part of the following composition:

C = 58·39 — 58·45
H = 6·90 — 7·19
N = 7·59 (Dumas).

It will thus be seen that under whatever name the substance now under discussion appears, whether as suprarenalin, adrenalin, etc., or in whatever manner it is prepared, its composition when properly purified is always found to be that given above by my own products:

Found for the Substance as Made by the Zinc-Ammonia Process.	Found as Made by the Trichloracetic Method.	Required for $C_{10}H_{13}NO_3\frac{1}{2}H_2O$.
C = 58·61 — 58·67	C = 58·72	C = 58·82
H = 6·84 — 6·77	H = 6·87	H = 6·86
N = 7·08 (Kjeldahl-Gunning).	N = 7·12 (Dumas).	N = 6·86
O = 27·47	O = 27·29	O = 27·56
100·00	100·00	100·00

The agreement between the found percentages and those required by the empirical formula $C_{10}H_{13}NO_3\frac{1}{2}H_2O$ are so close that we are justified in ascribing this formula to the above variety of epinephrin.

CONVERSION OF $C_{10}H_{13}NO_3\frac{1}{2}H_2O$ INTO THE ALKALOIDAL FORM, $C_{10}H_{13}NO_3$.

It was stated in the beginning of this paper that after finding that my first series of compounds ($C_{17}H_{15}NO_4$) had retained an unsaponified benzoyl radical, I was forced to adopt the formula $C_{10}H_{11}NO_3$ as the true empirical expression for alkaloidal epinephrin.

It now appears that the crystalline compound above described ($C_{10}H_{13}NO_3\frac{1}{2}H_2O$) is easily changed into the alkaloidal form $C_{10}H_{13}NO_3$ without first benzoating and then saponifying its benzoyl compound.

It is only necessary to dissolve it in concentrated hydrochloric acid or in very strong sulphuric acid in order to effect a dehydration and to give it the missing alkaloidal properties. Short exposure to very dilute mineral acids for a short time in the autoclave at pressures of 2 or 3 atmospheres also effects this dehydration. Furthermore, a brief exposure in vacuo of the thoroughly dry crystalline hydrate to a temperature of 177° also effects the dehydration, although it must be stated that this is not an economical method of conversion, inasmuch as secondary changes occur, the material taking on a brownish-red color and a certain amount of loss resulting.

I have thus far found solution in strong sulphuric acid to be the best means of effecting the dehydration. For example, 2·2 grammes of the pure crystalline hydrate analyzed above was dissolved in *small* portions at a time in 5 to 7 c.c. of sulphuric acid (prepared by adding 1½ c.c. of water to 16 c.c. of concentrated sulphuric acid), the process being a tedious one requiring several hours. After the solution has been effected it may be allowed to stand over night in the desiccator. The sulphuric acid solution is then dropped slowly into absolute alcohol, being stirred all the time.

A white precipitate immediately falls out, which may be further increased by the addition of a little ether.

After collecting the sulphate thus precipitated on the filter and washing it with absolute alcohol and ether, it is quickly dried in vacuo.

As thus thrown out in the amorphous form, this sulphate naturally retains some adherent sulphuric acid, which may, however, be removed with the greatest ease by dissolving it in a little water and dropping its aqueous solution into absolute alcohol under constant stirring. After being washed with alcohol and ether and dried in vacuo over sulphuric acid, this sulphate constitutes an almost white or grayish-white non-hygroscopic product, indistinguishable in appearance from the monobenzoyl sulphate of my earlier papers.

It dissolves very easily in water, dilute solutions being colorless, while very concentrated solutions take on a greenish-black color. Concentrated solutions of the hydrate ($C_{10}H_{13}NO_3 \frac{1}{2}H_2O$) in dilute acids take on a dark-brown color, while dilute solutions, 1 to 100 or 1 to 1,000, are also colorless.

No permanent salts of the crystalline hydrate have thus far been made. Takamine, for example, in attempting to make salts of the hydrate (adrenalin) obtained only noncrystallizable "brown brittle masses deliquescent in the air." The above dehydration, however, at once gives us a method for obtaining a whole series of permanent non-hygroscopic salts. There is every indication, too, that these can be obtained in the crystalline form. This sulphate, when thrown out of very dilute solutions in weak alcohol with ether, is deposited in microcrystalline form on the sides of the vessel. The picrate also has been obtained by me in the form of nodules made up of smal prisms, though not always perfectly formed.

This sulphate of alkaloidal properties thus prepared appears, as

far as I can now see, to exhibit all of the reactions of monobenzoyl-epinephrin sulphate as formerly described by me. I would also state that very dilute solutions of this alkaloidal sulphate give a green color on the addition of very dilute ammonia, while a similar solution of the hydrate, $C_{10}H_{13}NO_3 \frac{1}{2} H_2O$, takes on a pink color with the same treatment. Like the monobenzoyl compound, it is precipitated from solutions of its salts by ammonia in an amorphous form, easily soluble in excess of this reagent.

I would also add that after *complete dehydration the substance, in the form of the sulphate at least, has entirely lost its local vaso-constricting action.* That it is not, however, devoid of all physiological activity is to be seen in the work of Amberg.[1] A more complete pharmacological and therapeutical study of these alkaloidal salts of epinephrin is now in progress in my laboratory.

I will here offer the following analyses of the simple alkaloidal compound obtained by dehydrating the non-alkaloidal form, $C_{10}H_{13}NO_3 \frac{1}{2} H_2O$, with concentrated sulphuric acid. Specimen I was twice precipitated with absolute alcohol, dried in vacuo over sulphuric acid, then in vacuo at 65° C. in order to remove every trace of alcohol. Specimen II is like Specimen I; but in consequence of an accident in which concentrated sulphuric acid was spilled into it, it was again redissolved and reprecipitated three times in order to remove all traces of the acid. It was then dried in vacuo over sulphuric acid.

I. Found	II. Found.	Required for $(C_{10}H_{13}NO_3)_2 . H_2SO_4$.
C = 48·91	C = 49·05	C = 49·18
H = 5·58	H = 5·67	H = 5·74
N = 5·99	N = 5·905	N = 5·74
	H_2SO_4 = 20·59	H_2SO_4 = 20·08

Analytical data:

I. 0·1656 gramme substance gave 0·2970 CO_2 and 0·0831 gramme H_2O; therefore, C = 48·91 per cent. and H = 5·58 per cent.

0·3895 gramme gave 20·55 c.c. of dry N (collected over 50 per cent. KOH) at 24° C. and 751 mm. barometric pressure; therefore, N = 5·99 per cent.

II. 0·2079 gramme substance gave 0·3739 gramme CO_2 and 0·1060 gramme H_2O; therefore, C = 49·05 per cent. and H = 5·67 per cent.

0·4077 gramme substance gave 21·1 c.c. of dry N (collected over 50 per cent. KOH) at 23° C. and 752 mm. barometric pressure; therefore, N = 5·95 per cent.

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[1] *Archives internat. de Pharmacodynamie et de Therapie*, Vol. XI, 1902, p. 79.

0·1520 gramme substance dissolved in very dilute hydrochloric acid and precipitated with barium chloride gave 0·0746 $BaSO_4$; therefore, H_2SO_4 = 20·59 per cent.

It will thus be seen that a perfectly definite compound of the formula $C_{10}H_{13}NO_3$ *can be made in any desired quantity from the compound,* $C_{10}H_{13}NO_3{}^1/_2H_2O$. The dehydrated molecule still behaves in the same way toward ferric chloride, silver nitrate, copper sulphate, etc., but has taken on a new set of properties, viz.: alkaloidal characteristics. *As this substance, in the form of its monobenzoyl compounds, has long been known as epinephrin, it seems allowable, in view of the analytical proofs here furnished and the relationships that have been established, to give to the crystalline form,* $C_{10}H_{13}NO_3{}^1/_2H_2O$, *known in its less pure form as adrenalin, suprarenin, etc., the name epinephrin hydrate.*

The analytical data here given show that the formula $C_{10}H_{11}NO_3$, which I have long maintained as representing the true composition of alkaloidal epinephrin, is to be changed to $C_{10}H_{13}NO_3$. That I made this small error in the formula is due solely to the fact that my earlier preparations were, as a rule, dried at too high a temperature. I have observed, for example, that the sulphate just described when dried in vacuo at 100° loses hydrogen and shows a higher carbon content, and if then analyzed will apparently have the composition $(C_{10}H_{11}NO_3)_2H_2SO_4$, at least in respect to carbon, hydrogen and nitrogen.

The formula of monobenzoyl epinephrin must therefore also be changed from $C_{17}H_{15}NO_4$ — $(C_{10}H_{10}NO_3 . C_6H_5 . CO)$ to $C_{17}H_{17}NO_4$ — $(C_{10}H_{12}NO_3 . C_6H_5 . CO)$. At least one compound of the monobenzoyl series is stable enough to endure a drying temperature of 100° C., and in this instance it is found that no change in the formula need be made. I refer to the phenyldicarbamic ester of monobenzoyl epinephrin which was briefly described in an earlier communication.[1] This ester was made from the sulphate of monobenzoyl epinephrin, and the numerous chemical stages through which the compound was made to pass guarantees its chemical individuality and purity.

The following analyses of its sulphate further fortify the formula,

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[1] *Amer. Jour. of Physiology*, Vol. III, 1900; *Proc. Amer. Physiol. Soc.*, p. xvii; *Johns Hopkins Hospital Bulletin*, Vol. XII, 1901, p. 342.

$C_{10}H_{13}NO_3$, which I have now finally adopted for the basic substance in my whole series of epinephrin compounds:

Found.	Calculated for $[C_{17}H_{15}NO_4(CO \cdot NH \cdot C_6H_5)_2]_2H_2SO_4$.
C = 63·14	C = 63·48
H = 4·89	H = 4·78
H_2SO_4 = 8·46	H_2SO_4 = 8·36

Analytical data:

0·2887 gramme substance, dried at 100° C., gave 0·6684 gramme CO_2 and 0·127 gramme H_2O; therefore, C = 63·14 per cent. and H = 4·89 per cent.

0·2506 gramme dried at 100° C., analyzed by Liebig's method, gave 0·0505 gramme $BaSO_4$; therefore, H_2SO_4 = 8·46 per cent.

In concluding this part of my work I would add that the sulphate just described is insoluble in water and that other salts of the above ester are readily formed. Among these salts the picrate is notable in that it may be obtained in the form of large, thin crystalline plates, from concentrated solutions in very dilute alcohol. There are indications also that monobenzoyl epinephrin can take up a third molecule of phenylisocyanate ($CO : N \cdot C_6H_5$) and thus yield the phenylcarbamic tri-ester of monobenzoyl epinephrin.

NOTE ON THE BENZOYL COMPOUND OF THE CRYSTALLINE HYDRATE $C_{10}H_{13}NO_3\frac{1}{2}H_2O$.

It is apparent that derivatives of epinephrin may now be more easily and economically prepared by starting with the hydrate than by using the more cumbrous method that necessitates large quantities of gland extracts. I have repeated much of my earlier work, using the crystalline hydrate as the starting point, and have found that the methods formerly applied to extracts now led to identical results. In benzoating the hydrate I have found that the best method is to use powdered sodium carbonate as an alkali, adding it in proper amounts from time to time.

The white tarry benzoyl compound is easily purified by the methods already described. In saponifying the benzoyl compound I have found it best not to use sulphuric acid of more than ½ or 1 per cent., and not to let the temperature of the autoclave rise above 128° to 130° C. After exposing it to this temperature for two hours, it is best to remove the portion already decomposed and to add to the undecomposed cake of benzoyl compound a fresh portion

of the dilute sulphuric acid, and again to subject it for a couple of hours to the temperature of 128° C. Only a small portion of the undecomposed benzoyl compound now remains, and a repetition of the process would still further reduce this amount.

From the saponified portions, it is easy to prepare the picrate, bisulphate and other salts of monobenzoyl epinephrin, $C_{17}H_{17}NO_4$. That here, too, a single benzoyl radical is retained, just as in the earlier experiments with gland extracts, is easily proved. One has but to prepare the bisulphate and to treat it with concentrated sulphuric acid with gentle heat, dilute with water and shake out with ether in order to obtain benzoic acid as proof that a benzoyl radical had been retained. How many benzoyl groups can be taken up by the hydrate $C_{10}H_{13}NO_3{}^1/_2H_2O$ has still to be determined. It has been shown that alkaloidal monobenzoyl epinephrin can take up three additional acid radicals, but from this fact we are not justified in asserting that the crystalline hydrate can take up only four acid radicals.

ON THE BEHAVIOR OF EXTRACTS OF THE GLAND AND OF THE VARIOUS FORMS OF THE BLOOD-PRESSURE-RAISING PRINCIPLE TOWARD FEHLING'S SOLUTION.

In an earlier paper[1] I have pointed out that while *epinephrin in its native state* fails to reduce Fehling's solution, the monobenzoyl compounds and other products prepared by me agreed with adrenalin in their ability to reduce Fehling's solution. Certain preparations made in accordance with processes first developed by v. Fürth also did not in my hands reduce Fehling's solution; such were his iron compound and a very active amorphous and hygroscopic sulphate or bisulphate. In maintaining that *native epinephrin* as contained in more or less purified extracts of the suprarenal gland does not reduce Fehling's solution, I stood on common ground with Fraenkel, Moore, Metzger, and v. Fürth. I have also pointed out, however, that Brunner[2] takes the opposite view; that he has stated positively that an alcoholic extract of the gland does reduce Fehling's solution, *with a resulting deposition of cuprous oxide*. Aldrich[3] has also taken this position.

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[1] *Johns Hopkins Hospital Bulletin*, November, 1901.
[2] *Johns Hopkins Hospital Bulletin*, July, 1897.
[3] *Am. Jour. Physiol.*, Vol. VII, p. 359.

I have lately repeated a part of my work bearing on this question, and without entering into details I would say that I now find that a purified extract of the gland which contains only such principles as are soluble in strong alcohol and insoluble in ether behaves in the following manner toward Fehling's solution: if an aqueous solution of such an extract be poured into a goodly excess of Fehling's solution (Fehling 1 to water ½) and the mixture is kept at the boiling point for two minutes and then at once cooled down, no cuprous oxide settles out. A flocculent, greenish-white copper compound will, however, be found suspended in the fluid. After boiling from five to six minutes a considerable reduction occurs and yellow cuprous hydrate begins to be deposited in large amount. After boiling for fifteen minutes the reduction appears to reach a maximum, and a heavy deposit of cuprous hydrate, mixed with perhaps a trace of the red cuprous oxide, is obtained.

The extracts used in these experiments contained a large percentage of the active principle, the amount being in each case determined by precipitating the principle from a weighed amount of extract with ammonia or sodium carbonate, drying and weighing.

After removing the crystalline active principle from a given portion of extract with sodium carbonate, the filtrate, contrary to the statements of Aldrich, was always found to reduce Fehling's solution even in small amounts, provided only that the boiling was continued for from five to fifteen minutes as in the experiments with the original extracts. Such a filtrate is estimated, both by colorimetric and blood-pressure tests, to contain from 15 to 20 per cent. of the active principle, and the amount of reduction is apparently proportional to this unprecipitated part.

The ease and rapidity with which the active principle itself reduces Fehling's solution, with deposition of cuprous oxide, the reaction beginning far below the boiling point, is in great contrast to the behavior of the above extracts. These extracts also have no difficulty in reducing ammoniacal silver nitrate solutions. A fuller knowledge of the chemical composition of the gland, and especially of the exact form in which the blood-pressure-raising principle exists in the gland, will explain why an extract of the gland requires such prolonged boiling before the cuprous hydrate is deposited from the Fehling's solution.

I have observed that the blue color of a Fehling's solution is

immediately and entirely discharged when the solution is added in small quantities at a time to diluted extracts of the gland like those above described, but which are kept at the boiling point. In this way a considerable reduction of Fehling's solution without deposition of cuprous oxide may be obtained. The steam that arises from a boiling mixture of this kind is strongly alkaline, and this fact at once suggests that ammonia or some other basic substance prevents the deposition of cuprous oxide. We may here be dealing with something on the order of a Pavy-Fehling solution. The prolonged boiling is probably necessary in order to expel ammonia and to decompose substances that prevent the deposition of cuprous hydrate or oxide.

While now agreeing with Brunner and Aldrich that long boiling will develop the reducing power of a suprarenal extract for Fehling's solution, I would point out that Aldrich has not correctly reported my results. *He withholds the statement that all of my salts of epinephrin as obtained by saponifying its benzoyl or acetyl derivatives are as capable of reducing Fehling's solution as adrenalin itself, and thereby gives the reader the impression that no form of epinephrin thus far isolated is able to effect this reduction.*

It was stated clearly in my paper[2] that epinephrin in its *native unaltered state*, as found in extracts of the gland and in v. Fürth's lead precipitate, failed in my hands to reduce Fehling's solution, *but that my own compounds as obtained in various ways all agreed with adrenalin in their ability to reduce this reagent, that in this particular at least there was no difference between them.*

The suppression of these facts by Aldrich and his distortion of the point at issue are entirely unwarranted.

ON THE CHEMICAL CONSTITUTION OF EPINEPHRIN AND ITS HYDRATE.

I have repeatedly pointed out that epinephrin and its hydrate (adrenalin) behave in exactly the same manner toward the fixed alkalies, carbonates of the alkalies, and the hydroxides of the alkaline

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[1] See page 360 of Aldrich's paper, where he says: "I consider the failure of the various workers in this field to obtain a product capable of reducing Fehling's solution due to a changed form of the active principle, etc.," as also conclusion (6) of his summary.

[2] *Johns Hopkins Hospital Bulletin*, Vol. XII, 1901, pages 337-338; and conclusions 1, 2, and 3 of summary on page 343.

earths; that is, that on the addition of any of these to either modification of our substance, a volatile base of a coniin-piperidin-like odor is liberated, and in the course of time a pigment which I have named epinephrinic acid is also formed. This pigment I assume to be the oxidation product of the pyrocatechin-like part of the molecule. It was shown in my earliest papers that pyrocatechin cannot be split off from epinephrin by boiling with mineral acids of ordinary strength. In a later paper it was mentioned that when monobenzoyl epinephrin bisulphate is heated in a sealed tube with 25 per cent. hydrochloric acid to 150° C., a small amount of an ether-soluble substance is obtained which gives a fine green color on the addition of ferric chloride. V. Fürth has also found that on dry distillation a substance soluble in ether is obtained which behaves toward ferric chloride like pyrocatechin. Takamine[1] has obtained similar products by fusing adrenalin with caustic potash, but makes no reference to these earlier observations. From these observations it seems fair to conclude that there exists a residue $C_6H_3(OH)_2$—or $C_6H_2(OH)_3$ in the molecule of our substance. I had already expressed this opinion in my preliminary constitutional formula[2] for monobenzoyl epinephrin, $C_{17}H_{17}NO_4$, and it may be remarked that the subsequent discovery of an unremoved benzoyl group, C_6H_5CO, in this form of epinephrin does not invalidate the position taken by me at that time in respect to the pyrocatechin-like residue of the molecule.

Acting on the supposition that the molecule of the blood-pressure-raising principle consists of two cyclic compounds, one a hydroxylated benzene nucleus, the other a pyrrol-like base, I have lately undertaken a study of the action of nitric acid on both epinephrin, $C_{10}H_{13}NO_3$, and its hydrate.

Results thus far obtained indicate that the products of this oxidation are identical in the two cases. The following example will illustrate the process: In very small portions at a time, 10 grammes of the purified hydrate is dissolved in 60 c.c. of nitric acid, of sp. gr. 1·2, in a platinum bowl on the water-bath. The oxidation goes on with considerable violence, and care must be taken to avoid loss of material in consequence of foaming. When the evolution of

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[1] Am. Jour. Pharmacy, Vol. 73, 1901, page 529.

[2] See *Zeitschr. f. Physiol. Chem.*, Bd. XXVIII, page 347.

gases has largely subsided, 10 c.c. of fuming nitric acid is added and the whole is then concentrated on the water-bath, water being added from time to time as the mass begins to thicken. After the removal of excess of acid and cooling, the contents of the dish are found to be crystalline and odorless.

The larger part of this crystalline mass consists of oxalic acid. The barium, lead, sodium and calcium salts of this acid were prepared, as also its diethyl ester. The calcium salt $CaC_2O_4 . H_2O$, as prepared from a hot concentrated solution of the sodium salt, was found to contain 27·39 per cent. Ca, the theoretical amount of calcium for $CaC_2O_4 . H_2O$ as thus prepared. The tetragonal crystals, $CaC_2O_4 . 3H_2O$, were also prepared from the sodium salt and the free acid was oxidized with potassium permanganate, and in other ways was shown to have all the properties of oxalic acid.

The other chief product of the above oxidation consists of a salt (oxalate?) of the unknown nitrogenous base which I have called the coniin-piperidin-like body on account of its peculiar offensive and penetrating odor. The addition of an alkali to these crystals immediately liberates this odor. It is evident, then, that this part of the molecule has withstood the very energetic treatment with nitric acid above described. Further treatment with iodine trichloride does not destroy this base, but enables one to obtain it as a salt crystallizing in slender prisms, very soluble in water and alcohol and but little soluble in ether. A diazo-compound of these crystals has also been formed.

In its general behavior toward destructive chemicals, as, for example, on fusion with powdered potassium hydrate, this salt reminds one forcibly of the behavior of certain pyrrol derivatives under similar circumstances. When thus treated an odor like that of pyrrolidin arises; later, as the fusion continues, this gives place to the fishy odor of amines, and this in turn gives place to that of pyrrol, as proved by the pine sliver reaction. Analyses of this specific part of the molecule of epinephrin and adrenalin will soon be published.

It is evident from the above that the pyrocatechin-like part of the molecule, which in some measure resists the destructive action of acids in sealed tubes and of fusion with alkalies, is oxidized by nitric acid to oxalic acid and simpler products, while the nitrogenous part of the molecule remains, in large part at least, entirely unaffected by this oxidation. Researches now in progress will, it is hoped, soon

throw more light on the constitution of this interesting nitrogenous derivative.

It has been shown that the alkaloidal modification of our substance can take up four acid radicals. This is proved by the fact that monobenzoyl epinephrin, $C_{17}H_{17}NO_4$, is capable of taking up three acetyl groups. Inasmuch as this monobenzoyl compound was formed by benzoylating the native principle as existing in extracts of the gland, it may be assumed that epinephrin hydrate also is capable of taking up at least four acid radicals. This point is now being put to the test by experiment. The above experiments, nevertheless, show that we already have some little insight into the chemical character of this interesting compound.

A NOTE ON THE QUANTITATIVE ESTIMATION OF PHOSPHATES IN STOMACH CONTENTS.

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Having had occasion in the course of the last two or three years to make a large number of analyses on normal and pathological stomach contents, I have had the opportunity of remarking how absolutely unreliable are the various volumetric estimations, and further, that the ideas generally held regarding the significance of individual titrations are frequently erroneous. It has been found necessary in our work on metabolism to make gravimetric determinations of the various constituents wherever the stomach contents were concerned. I do not propose in this paper to deal with the various phases of this subject, which will be reserved until the completion of some work now in progress on cancer of the stomach; but simply with the estimation of acid phosphates, which affords a good illustration of the errors of volumetric analysis.

Phosphoric acid is tri-basic; that is to say, is possessed of three hydrogen atoms capable of being displaced by monovalent bases, as sodium, potassium, etc. Each of these hydrogen atoms can be saturated separately and the end point of each may be very readily determined as follows:

A solution of phosphoric acid is prepared and titrated by means

of one-tenth normal soda, different indicators being employed in order to determine the point of saturation of the individual acid groups.

(1) Phosphoric acid is completely converted into mono-sodium phosphate, NaH_2PO_4, at the alizarin end point.

(2) The acid is completely converted into di-sodium phosphate, Na_2HPO_4, at the phenolphthalein end point.

(3) The acid is completely saturated with the formation of Na_3PO_4, or its equivalent, when an excess of neutral barium chloride[1] and a definite excess of the one-tenth normal alkali employed are added to the solution, which is boiled, and the residual excess of alkali titrated by means of one-tenth normal acid, phenolphthalein still being employed as indicator.

If a solution of phosphoric acid is prepared of such a strength that, using alizarin as indicator, 10 c.c. are exactly equivalent to 10 of one-tenth normal alkali, then 10 c.c. of the same solution would require 20 of the alkali, using phenolphthalein as indicator, and 30 using phenolphthalein in the presence of an excess of neutral barium chloride. As regards other indicators which have been carefully tested, tropæolin and phloroglucin vanallin give practically no reaction. Dimethyl-amido-azo-benzol gives an indefinite end point, starting shortly after the commencement of the titration and reaching the green stage shortly before the alizarin end point. Methyl-orange, benzo-purpurin and congo give fair end points in the neighborhood of the alizarin end point. Litmus, lakmoid and rosolic acid give indefinite end points, somewhere in the neighborhood of the phenolphthalein end point. In the presence of barium chloride, rosolic acid is affected in just the same manner as phenol phthalein, giving an indication at the end point, Na_3PO_4. We should not, however, recommend any of these indicators for use in estimation of phosphates, as alizarin and phenolphthalein have been found to give excellent results when employed in the manner which will be described later.

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[1] The explanation of this reaction is as follows: Na_2HPO_4 is neutral to phenolphthalein, but Na_3PO_4 is alkaline. The addition of neutral barium chloride to the solution leads to the immediate precipitation of neutral barium phosphate, $Ba_3(PO_4)_2$, so soon as formed, in such a manner that final end point with phenolphthalein is obtained on complete saturation of the phosphoric acid. See "Sutton's Volumetric Analysis."

If we now turn to a consideration of the methods usually employed in analyzing stomach contents, and remember that the difference between the dimethyl-amido-azo-benzol end point and the alizarin end point is usually considered as due to acid phosphates and organic acids, whilst the residual portion of the titration from alizarin to phenolphthalein is not usually considered to be in any sense due to phosphates, but entirely to combined hydrochloric; it is obvious that some discrepancy exists. The results of our analyses with normal phosphates and phosphoric acid, checked by gravimetric methods, have proved conclusively that two of the three available acid groups of the phosphoric acid are neutralized in the course of the titration of stomach contents up to the phenolphthalein end point; that one only of these is neutralized at the alizarin end point; that is to say, that one of the three acid equivalents of the phosphates present in stomach contents is continually mistaken for combined acid, whilst the difference between the dimethyl-amido-azo-benzol and alizarin end point is seldom sufficient to account for even one of these groups, since dimethyl-amido-azo-benzol always reacts to a certain extent to phosphoric acid and the alizarin end point is sharp on saturation of one acid affinity.

Realizing this discrepancy and finding that it interfered seriously with a long series of comparative experiments which have been carried out in the laboratory regarding chlorides, nitrogen, etc., of stomach contents, we at first attempted to estimate the phosphates gravimetrically and volumetrically by means of uranium nitrate, subsequently allowing for the effect produced by the known quantity of phosphoric acid, but the amount of available material is usually so small as to render either of these methods extremely unsatisfactory, and we were eventually led to adopt the following method, which is comparatively simple and fairly accurate, affording, as it does, a means of checking one's results by duplicate experiments:

As large a quantity of the contents as possible, in no case less than 20 c.c., is evaporated to dryness in a platinum dish and gently incinerated in order to destroy organic acids. The residue is extracted with a small amount of dilute sulphuric acid free from phosphates; made up to 25 c.c., two batches of 10 c.c. each being employed for the analyses, which are carried out in the following manner: (1) Under ordinary circumstances, a one-tenth normal solu-

tion of alkali and acid may be employed, but under exceptional circumstances, where the phosphates are known to be present in very small quantities, very satisfactory results may be obtained by using one-fiftieth normal solutions. Ten cubic centimeters of the solution obtained above, containing phosphates, is exactly neutralized with one-tenth normal alkali to the phenolphthalein end point. Alizarin is then added and the mixture is titrated back with one-tenth normal sulphuric until the alizarin end point is reached. This figure represents the equivalent of one of the acid groups of the phosphoric acid. (2) Ten cubic centimeters are neutralized in the same way as the previous experiment, using phenolphthalein as an indicator, an excess of 2 or 3 c.c. alkali is then added and the mixture boiled. It is then retitrated with sulphuric acid to see if any appreciable loss of alkali has taken place. If the difference is more than 0·1 of a c.c. a further excess of alkali is added, once more boiled and retitrated to the neutral point with sulphuric until fairly constant. Then a definite excess of alkali, 5 c.c. for example, is added to the mixture, and 5 c.c. of an absolutely neutral and phosphate-free barium chloride solution, the whole is boiled vigorously for a minute, allowed to cool for a minute, and titrated with one-tenth normal acid until the phenolphthalein end point is reached. If phosphates are present it will be found that the full amount of acid is not required. The difference between the amount of alkali added and the amount of acid required to titrate back represents another acid group of the phosphoric acid. This figure should exactly coincide with that obtained above in the first experiment, since each one of them represents one of the three acid affinities of the total amount of phosphates present in the mixture. As a matter of fact, the phenolphthalein alizarin end point is more nearly correct, the other result being usually slightly high. If any great discrepancy exists it is necessary to look for some source of error and repeat the experiments. Having obtained these results it is comparatively easy to calculate the exact effect which would have been produced by the phosphates present in the mixture upon the alizarin end point and the phenolphthalein end point, allowance being made accordingly. It is obvious that the effect upon the alizarin end point would be exactly half that upon the phenolphthalein end point, since one hydrogen was saturated at the alizarin end point and a second at the phenolphthalein end point.

It may further be noted for the benefit of those who have not platinum vessels at their disposal, and who have to consider the factor of time, that a fairly accurate estimate of phosphates may frequently be made in the following manner: After titrating the stomach contents, using phenolphthalein as indicator (in which experiment as large a quantity of stomach contents as possible should be employed), an excess of three or four cubic centimeters of alkali is added, the mixture boiled vigorously and neutralized with sulphuric acid as in the above experiments. A known excess of alkali is then added, and the five cubic centimeters of neutral barium chloride solution; the mixture once more boiled and titrated with one-tenth normal acid. The difference between the amount of alkali employed and the amount of acid required for titrating back gives the effect due to the third acid affinity of the phosphoric acid between Na_2HPO_4 and Na_3PO_4, and is equal in quantity to that exerted by the phosphates on the alizarin end point for the same quantity of material from H_3PO_4 to NaH_2PO_4. It also represents half the effect on the phenolphthalein end point from H_3PO_4 to Na_2HPO_4, and is equal to that between the alizarin and phenolphthalein end point, NaH_2PO_4 to Na_2HPO_4, and, therefore, has to be deducted from that quantity in order to obtain a more correct estimate of the so-called combined hydrochloric.

In making use of this method without evaporation and ashing, it must be remembered that the phenolphthalein end point is seriously interfered with and that certain other disturbing factors prevent as much accuracy as is desirable, but still it affords much better results than are obtainable either by means of uranium nitrate in the stomach contents directly, a method which we employed for a considerable period of time, or any of the methods previously suggested, such as that obtained from the difference between the dimethyl amido-azo-benzol and alizarin end points, which, as we have shown above, is absolutely incorrect.

An illustration of our method may make it easier to follow. Fifty cubic centimeters of filtered contents were evaporated to dryness, ignited, and extracted with boiling water, with addition of a small amount of acid. The whole was made up to 25 c.c. Two lots each of 10 c.c. were employed for the titrations, which were carried out as directed above.

(1) Phenolphthalein end point to alizarin end point = 0.8 c.c. n/10 acid.

(2) Difference between phenolphthalein end point and phenoltalein end point in presence of $BaCl_2$ = 0·85 c.c. n/10 acid, from which phosphoric effect for 50 c.c. of stomach contents employed is altogether equivalent to 4·12 c.c. of a one-tenth normal acid, and is made up as follows:

From alizarin to phenolphthalein end point, = 2·0 c.c.

From phenolphthalein to phenolphthalein $BaCl_2$ end point, = 2·12 c.c.

Consequently the effect on 100 c.c. of stomach contents would be respectively 4 c.c. and 4·24 c.c., total 8·24 c.c., which means that for 10 c.c of contents, the effect attributable to phosphates would be, in this case, 0·41 before the alizarin end point, and 0·41 between alizarin and phenolphthalein end points, giving a total effect of 0·82, on a total acidity of 4.5 c.c. This result was confirmed by means of uranium nitrate standard solution.

Summary.—It has been shown that the usual method of roughly estimating the effect due to acid phosphates in the titration of stomach contents is absolutely incorrect; that one of the three acid affinities of phosphoric acid is saturated at the alizarin end point, and a second at the phenolphthalein end point. Consequently less than one-half of the total effect due to phosphates lies between the diazo (so-called) and alizarin end points; whilst at least an equal effect is exerted on the portion usually looked upon as combined HCl.

A comparatively simple method has been suggested whereby a fairly accurate estimate of the phosphates present and the effect which they exert may be directly determined, dependent

(1) Upon the transition from NaH_2PO_4 to Na_2HPO_4 between the alizarin and phenolphthalein end points;

(2) The transition from Na_2HPO_4 to Na_3PO_4 between the phenolphthalein end point alone and in the presence of $BaCl_2$, whereby the alkaline Na_3PO_4 is converted into $Ba_3(PO_4)_2$ so soon as found and thus removed from the scene of action.

Incidentally, one object of this paper is to draw attention to the gross inaccuracies in the accepted methods of titrating stomach contents.

ALKALOIDAL COLOR TESTS, WITH THOSE OF STRYCHNINE AS EXAMPLES.

By Lyman F. Kebler,
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Before taking up these tests, a brief review of the general methods for recovering the alkaloids from organic mixtures might be of service.

J. S. Stas,[1] of Brussels, in 1851, first pointed out the underlying principles for isolating alkaloidal bodies in general. By this method alkaloids are removed by treating the organic matter with pure concentrated alcohol, then render distinctly acid with tartaric or oxalic acid, heat to about 70° C., cool the mixture, filter, wash the residue with alcohol and evaporate the mixed filtrates to dryness, at a temperature not exceeding 35° C. The residue is then digested with absolute alcohol, the filtrate evaporated at a low temperature, the resulting residue taken up in a small quantity of water, the solution treated with a slight excess of powdered sodium carbonate, and finally violently agitated with four or five volumes of pure ether. This operation dissolves the alkaloids in a fairly pure state. Separate the ether and evaporate spontaneously on a watch glass as occasion requires. Volatile alkaloids must first be converted into stable salts before the ether is dissipated.

For the extraction of many of the vegetable alkaloids, the above method is quite applicable, but the operator must bear in mind three points: (1) An alkaloid extracted by the above process is very liable to be contaminated with coloring matter and certain plant principles; (2) that some of these alkaloidal bodies are quite insoluble in ether; consequently, with such chemicals the results are either only partially successful or fail entirely; and (3) the influence of the presence of one body on the solubility of another.

The first difficulty was partially met by F. J. Otto,[2] who treated the acidulated solution, containing the alkaloid in the form of a salt, with ether, in which alkaloidal salts in general are insoluble, thus removing undesirable bodies from the alkaloidal solution. After the above treatment, the solution is rendered alkaline with a fixed

[1] *Bull. de l'Académie de Médécine de Belgique*, 9, 304; *Ann.* (Liebig), 84, 379.

[2] 1856, *Ann.* (Liebig), 100, 39.

alkali, and the liberated alkaloid removed by ether as in Stas' process. It must be borne in mind, however, that in treating the acidulated solution with ether, certain alkaloidal bodies, as well as coloring matter and other associated impurities, may be removed. This is especially true of the weaker bases, such as berberine, hydrastine, colchicine, etc.

The second difficulty was overcome by the introduction of chloroform[1] and amylic alcohol.[2] Chloroform and ether are usually employed because they volatilize readily at low temperatures, and most alkaloids are sufficiently soluble, for most work, in either one or the other or mixtures of the two. Amylic alcohol is usually employed only for morphine, which is quite insoluble in the above solvents.

The third point has never received much attention, consequently our knowledge along this line is very fragmentary. There are many cases where a substance is insoluble or sparingly soluble in a given solvent, but the presence of a third body makes it freely soluble. For example, iodine, sparingly soluble in water, is rendered freely soluble in the presence of potassium iodide or certain other alkaline halogen compounds. Some of the metallic cyanides are insoluble in water, but these are rendered freely soluble by the presence of an alkaline cyanide like potassium cyanide. Bismuth citrate is insoluble in water, but is freely soluble in this menstruum containing alkaline citrates. Pure water dissolves only traces of the essential oils, whereas, concentrated aqueous saccharine and sodium salicylate solutions have the power of dissolving considerable quantities of these oils. Castor oil is practically insoluble in petroleum ether, but this characteristic insolubility is lost at the ordinary temperature, when castor oil is mixed with an oil soluble in the above solvent, like croton oil.

G. Dragendorff[3] introduced a most comprehensive scheme, which includes all of the good points of his predecessors' methods, by not only increasing the number of immiscible solvents employed, but also including glucosides, other indifferent plant principles, and some synthetic remedies. Ingenious and comprehensive as is this scheme, yet very few, if any, of the many organic bodies it is

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[1] 1856, J. E. D. Rodgers and G. P. Girwood, *Lancet*, June, 718; *Pharm. J. Trans.*, 16, 497.

[2] 1861, L. Uslar and J. Erdmann, *Ann.* (Liebig), 120, 121.

[3] 1867, *Pharm. Ztschr. f. Russ.*, 6, 663; *Ztschr. anal. Chem.*, 7, 521.

designed to separate are wholly insoluble in the different solvents employed; and unless a body is present in considerable quantities, it might be removed completely from the aqueous solution before the best conditions for its recovery were reached. For example, morphine and its sulphate are not absolutely insoluble in the seven solvents employed, to about exhaustion, before treating the mixture with the solvent in which the alkaloid is most soluble, viz., amylic alcohol. It may be possible that sufficient morphine is present to carry it through these extractions, yet the chances are decidedly unfavorable. If in this connection we consider the influence of one body on the solubility of another, it can readily be seen how incomplete the separations must be even with this excellent method. That such influences exist in the organic world can be shown by numerous instances. Take, for example, a simple solution containing morphine and hydrastine sulphate, render alkaline and extract with ether, evaporate the ethereal solution to dryness, and it will be found, contrary to expectations, that sufficient of the morphine is removed with the hydrastine so that the color reactions of the residue are quite different from those obtained for pure hydrastine. Consequently, it seems to the writer that the toxicologist should never employ this method, in all of its details at least, unless there is absolutely no clue whatever as to the general nature of the poison from either symptoms or circumstantial evidence, which very rarely is the case.

T. Graham[1] introduced a method in which he utilized the remarkable property that moist organic membranes possess, of allowing crystallizable bodies in solution to pass through them, whereas, the non-crystallizable mostly fail to pass. Graham and Hofmann[2] devised a process, supposed to be especially adapted for the isolation of strychnine from certain organic substances like beer. In this method advantage is taken of a property charcoal possesses of absorbing strychnine from an aqueous solution and giving it up again to boiling alcohol. These methods are seldom used at present, because small quantities of strychnine may escape detection, or will be so contaminated with foreign matter as to require numerous extractions with ether or chloroform, to render the alkaloidal material pure, so that it would be far more expedient to begin the

[1] 1862, *Jour. Chem. Soc.*, 15, 216.
[2] 1853, *Quart. Jour. Chem. Soc.*, 5, 173.

ethereal or chloroformic extraction directly with the original material.

F. Selmi[1] further purifies the alkaloids, as usually obtained in the ethereal extractive, by precipitating them from this ethereal solution, both with and without an excess of water, by means of carbon dioxide. This method is said to possess certain merits, but on account of the uncertainties usually encountered, the method has gained very little prestige.

Notwithstanding such elaborate and, apparently, faultless schemes, this department of chemistry presents many complex problems. When it is remembered that nearly 2,000 substances, with definite chemical individualities, have been separated from plant tissues, and there is scarcely a line of demarcation anywhere between them, but they imperceptibly grade into one another; then add to this the host of synthetic bodies, which are employed as medicinal remedies, the difficulties to be encountered in isolating in a pure state, even some of the most characteristic ones from a heterogeneous mixture, becomes quite apparent.

ANALYTICAL REACTIONS OF STRYCHNINE.

Most alkaloids are precipitated from aqueous solutions, acidulated with a mineral acid, by a number of reagents, such as Mayer's, Wagner's, Scheibler's, Sonnenschein's, etc., and they serve very well as general reagents for establishing the presence of alkaloids; but further than this, little has been done with these reagents which is of distinct service in establishing the individuality of an alkaloid either qualitatively or quantitatively. We must, therefore, look for other information leading up to the identification of the various alkaloids, and this has been largely supplied by way of color reaction. But can these color reactions be utilized as the basis of a positive opinion? Blyth says, in substance, fairly pure alkaloids give certain color reactions more or less characteristic, but they are generally untrustworthy and must be looked upon as useful guides only, to be confirmed by other characteristics of the substance in quest. There are probably very few, if any, of the many alkaloidal color reactions that can be ascribed to definite chemical changes. In many cases, the color reactions are probably due to the presence of minute quantities of associated impurities, rather than the pure material itself. Again, the various shades of color are usually difficult to

[1] 1877, *Gazett. Chim. Ital.*, 6, 153; *Jour. Chem. Soc.*, 1, 93.

describe, and the color frequently changes from one shade to another so gradually that mistakes may easily arise. Even in the inorganic world, where certain colors are developed as the result of chemical combinations, we seldom trust ourselves with such information as positive; for example, in precipitating a soluble compound of arsenic with a sulphide, we have produced an almost infallible indication that arsenic is present; but what chemist is there, who would, without further submitting this precipitate to the other searching tests that we possess, for absolutely identifying the presence of arsenic, say that arsenic is present, without a question of doubt, simply from the color produced as the result of the formation of a chemical compound. Again, on passing hydrogen sulphide gas into a solution of mercuric chloride, we have formed, as we think, a definite chemical compound, and yet, who has not seen the various shades of precipitate that result when the above operation is performed?

The quality of the reagents seems to have been lost sight of in a large measure. It is an open secret that pure and even C. P. molybdic acid, the chief constituent of Fröhde's reagent, contains as much as 15 per cent. of ammonium nitrate and sulphate or the corresponding salts of sodium. Recently quotations were seen for C. P. phosphomolybdic acid, varying from $1.45 to $11.00 per pound. These examples incite thought. Do the reagents employed by the various workers the world over vary in quality as the above prices? How are the color reactions of Fröhde's reagent affected by the impurities of molybdic acid?

Within the last quarter of a century there have been isolated a large number of ptomaines, some of which give color-reactions similar to those of certain alkaloids.

In connection with the above we must not forget to mention imperfection of vision (color-blindness), which plays such an important part in the affairs of mankind. In general, therefore, color reactions can be considered useful only as guides and should never be considered conclusive in themselves.

Strychnine is one of the few alkaloids that toxicologists and alkaloidal chemists have pointed to with some gratification as being singularly invulnerable to the attacks made on color reactions from time to time, it being characterized by an infallible display of successive color reactions; and the writer believes that the successive color

changes for strychnine are perfectly reliable when applied to pure material; but in the former part of this paper there are pointed out some of the difficulties to be encountered in isolating and rendering absolutely pure minute quantities of any alkaloid. Not only is it difficult to purify small quantities of strychnine, but there may be present in the ethereal or chloroformic extracted material certain undefined organic substances which will interfere so seriously with the test for strychnine that its presence cannot be recognized even when comparatively large quantities of the alkaloid are present or are purposely added.

Evidence as to the presence of strychnine depends on joining the following results: (1) No coloration with pure concentrated sulphuric acid; (2) a purple-blue color when oxidation results; (3) fading of the purple-blue color to a bright cherry-red tint, etc.

There are very few organic bodies, usually encountered in the search for an alkaloid, that will comply strictly with the first requisite. Among these may be mentioned gelsemine, acetanilid, antipyrin, salicylic acid, benzoic acid and curarine, the non-crystallizable principle of worara, obtained from botanical sources allied to those of strychnine. Among the above are frequently mentioned brucine and morphine, and occasionally hydrastine. The two former usually produce a slight rose tint, while the latter a faint yellowish coloration. After dissolving the strychnine residue in sulphuric acid, the mixture ought to be allowed to stand five minutes in order to note if any color is developed.

On treating strychnine, dissolved in concentrated sulphuric acid, with almost any oxidizing agent, there is produced a rich purple-blue, which changes more or less rapidly through purple and crimson to a bright cherry-red tint, this latter being somewhat persistent. The rapidity of the change is largely influenced by the amount and nature of the oxidizing agent employed. Many oxidizing substances have been recommended from time to time, and each operator seems to have a distinct preference for one or the other, probably because of a greater familiarity with the same.

E. Marchand,[1] in 1843, showed that when strychnine, dissolved in sulphuric acid containing a little nitric acid, is treated with a small amount of lead peroxide, a series of colors was developed. Mack,

[1] *Jour. de Pharm.*, 4, 200.

in 1846, proposed the use of manganese dioxide, and about the same year Otto brought forward the use of potassium bichromate. Since then, potassium ferricyanide, ammonium vanavate, and cerosoceric oxide have all been ardently advocated. Potassium bichromate is the favorite of many workers; but on account of the rapid change of colors it produces, and the resulting green, chromium coloration, others prefer lead or manganese dioxide, both of which produce a remarkably well-developed play of colors.

As can readily be imagined, one or more of the successive colorations produced in the series of color reactions for strychnine can easily be imitated, with a number of organic bodies, under the same conditions, such bodies are acetanilid, anilin, antipyrin, curarine, cod-liver oil, gelsemine, pyroxanthine, papaverine, narceine, solanine and veratrine. In regard to these it may be said in general that there is more fiction than truth about these substances interfering with the identity tests for strychnine. These reactions are usually classed as fallacy tests.

It is the common belief that most of the above substances, that may interfere with the tests for strychnine, will be removed from the strychnine by the conventional methods of isolation; but it must not be forgotten that the line of separation is very seldom so complete as to entirely eliminate all associated impurities, except when sublimation can be resorted to according to Helwig's[1] scheme, subsequently improved by Drs. Guy[2] and Wormley[3] and frequently used at the present time.

Within a few years there has come to our attention a mixture of morphine and hydrastine, which is supposed to give color reactions simulating those of strychnine. Curious enough, it has come to us in the form of fiction, "Stringtown on the Pike," by J. U. Lloyd, and, as can readily be imagined, has created some little stir in certain circles. The writer has carefully gone over these tests, and finds that, while there is a similarity of color reaction, when the above mixture is treated with the usual reagents employed for identifying strychnine, yet when the first of the above propositions laid down is applied to this mixture, it will be found that it will not dissolve without coloration in sulphuric acid, which immediately elimi-

[1] Das Mikroscop in der Toxicologie.

[2] 1867, *Pharm. Journ. Trans.* (2), 8, 719, and 9, 10 and 58.

[3] Micro-chemistry of Poisons.

nates it as possibly being strychnine. We must not, however, forget that it is frequently impossible to purify strychnine to such an extent as to produce an absolutely colorless solution in sulphuric acid, and, if the remaining portion of the color reactions would develop properly, the chemist might conclude that he was justified in calling it strychnine. It should be stated here that when strychnine, dissolved in sulphuric acid, is treated with a fragment of potassium bichromate, a violet-blue streak follows the crystal, as it is moved about, whether the moving is slow or rapid, but the color is transient and changes slowly to orange. When a mixture of morphine and hydrastine is similarly treated, a greenish-yellow streak at first results, changing rapidly to purplish-violet, and finally a dirty chrome-green results. The shades of color produced with strychnine and morphine-hydrastine are not identical, but resemble one another so closely that it would be difficult to describe them so as to give a definite idea of their differences. .

Recently[1] A. H. Allen and G. E. Scott-Smith reported that the mixed alkaloids, as ordinarily removed from liquid extract of ipecac, gave color reactions similar to those obtained from opium bases. How many cases of this kind will be met in the future no one will venture to predict.

In view of what has been said above, and the fact that many of the color reactions proposed from time to time are short-lived, it behooves us to use them with the greatest circumspection; calling to our aid every possible assistance, such as the microscope and physiological tests.

THE FATHER OF AMERICAN PHARMACY.

By John F. Hancock.

There have been, and are now, many distinguished pharmacists in America, but the above title, won by the late Prof. Wm. Procter, Jr., has never been disputed; and for some time past it has been the purpose of those who have known him, both personally and through his works, to give him appropriate honor. Several methods have been suggested to give expression to that respect in which we all hold him, and while all have been well intentioned and sincere, it seems proper that we should be selective in the tribute that we would employ, and above all bestow that which is fittest.

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[1] 1902, *Analyst*, 27, 345.

Prof. Wm. Procter, Jr., occupied an unique position in the profession of pharmacy and, under great disadvantages, rose to the highest eminence. Beginning, with limited means, as an apprentice in an apothecary shop, without the advantages of a liberal education, he ascended, by diligence and well directed effort, from its rudiments to the top. In theory and practice he became the most accomplished pharmacist of his day and generation, and perhaps contributed more to the higher branches of pharmaceutical literature than any other pharmacist of the nineteenth century. His work was thorough and original, and evidenced the imprint of his character for systematic research; and the pages of the AMERICAN JOURNAL OF PHARMACY, beginning with his thesis for graduation from the Philadelphia College of Pharmacy in 1837, and continuing with regularity to the year of his death, attest his purpose in life and form a monument built by his own hands that will always be a beacon light to those who wish to enter the profession and who are interested enough to care for its scientific evolution. The proceedings of the American Pharmaceutical Association are also periodic witnesses to his industry and ability, both as an investigator and a writer; and at every annual meeting he contributed papers that were of practical utility to every pharmacist. At the last meeting that he attended—1873—a few months before his death, he contributed papers and took an active interest in the business of the association.

Professor Procter was successful in the respect, confidence and esteem of his associates, and in return was just, honorable and upright in his relations with his fellow-men. He loved knowledge and sought it, and in his benevolence willingly imparted, what he had so arduously learned, to others.

These are the monuments which he himself has builded, and it would now become us who have profited by his labors to make a substantial recognition to his memory, for, above all others of his class, his example is most worthy of emulation and lasting renown.

The American Pharmaceutical Association has taken a step in this direction, and at the semi-centennial meeting in Philadelphia last September a report was presented by the Committee on the "Procter Memorial" that was favorably received. Without meaning to object in any way to the report of this committee, I feel that something more should be done to honor and perpetuate the memory of this great and good man; a something that will substantially

show to the people of this country the regard that we have for him as our common preceptor, and which may be to them an object lesson in that loftier patriotism that is even beyond the deeds of warriors and statesmen—the preservation of the race.

From some has come the objection that a monument in bronze does not comport with the unostentatious life of Wm. Procter. Granted; but were that a precluding objection there would never have been a monument erected to the truly great, for this blessed condition is only attained by those who forget themselves in their work, and who are wise enough to not waste and dwarf their energy by display. The true heroes never work for the applause of the multitude, but

> "Bounded by themselves and unregardful
> In what state God's other works they see,
> In their own tasks all their powers pouring,
> These attain the mighty life we see."

Under the circumstances the committee has done its best and has acted within its scope; but I would now propose another plan, independent of the existing fund, previously known as the life-membership fund, and now by its advice to be named the Wm. Procter Fund.

To perpetuate the name and fame of Wm. Procter, Jr., there should be erected at Washington, D. C., his statue in bronze on a granite base of appropriate design and embellishment, and in accordance and plan, for instance, with that erected to the memory of the late Prof. Saml. D. Gross, the American Surgeon, by the physicians of this country. Such an honor paid to one of national character and reputation should be located at the national capital, and there is no doubt but that a suitable site could be readily obtained on the Smithsonian grounds; and it is even probable that the Government would desire to assist in this honor to one of her noble sons, and appropriate some portion of the expense.

The real fund should be open to subscriptions from the pharmacists of the country, in whatever amounts they may each wish to contribute, so that they may all feel that they have an interest in honoring one who has been a benefactor to the entire profession.

The American Pharmaceutical Association, being the parent body and entirely responsible, should take the initiative and be the custodian of this Monument Fund, and they should invite the co-operation

of the various State Associations and whoever else that have an interest in pharmacy and wish to express it.

The Maryland Pharmaceutical Association is already on record as favoring the monument and I feel that the other associations will be glad to act in harmony, and by this united and national action, comparatively small contributions will not only suffice, but each subscriber will feel his personal interest.

This would be a substantial and lasting recognition of the Father of American Pharmacy, which could not have been expected from the recommendations of the Procter Memorial Committee, and its fulfilment will redound to the glory of the American pharmacists—who will erect this memorial—apart from any particular association or associations.

BALTIMORE, June 12, 1903.

SOME OF THE UNPUBLISHED RESULTS OF THE INVESTIGATIONS OF THE TANNINS BY THE LATE PROFESSOR HENRY TRIMBLE.

BY W. E. RIDENOUR.

The work herewith recorded was carried on during the years 1894–95 under the direction of Professor Trimble.

The hide-powder method of estimating the tannin was used exclusively.

PINACEÆ.

Tsuga Canadensis.—The materials for this work were collected at Wissahickon, Pa.

Part.	Date of Collection.	Moisture.	Ash on Dry Basis.	Tannin on Dry Basis.	Remarks.
Whole bark	May 28, '94.	45·78	1·51	7·74	Tree about 30 ft. high.
" "	" "	42·72	1·46	5·46	" " 10 " "
" "	July 18, '94.	12·30	2·15	14·00	
Inner "	Aug. 16.	48·51	3·22	13·30	
Whole "	" "	8·63	1·83	19·93	
" "	Sept. 16.	8·87	2·86	11·66	Very hard to peel.
" "	Oct. 16.	10·52	3·11	11·75	
" "	Dec. 22.	31·92	2·17	10·42	
Inner "	Feb. 23, '95.	32·55	2·65	9·56	Sap beginning to flow.
Whole "	March 30.	38·31	1·93	10·25	Highland, Pa.

The separation and purification of the tannin was effected as follows: The ground dried bark was percolated with acetone; the solvent recovered and the residue treated with water. The solution was filtered clear and shaken with acetic ether, and as this did not remove the tannin, the watery solution was saturated with salt and again shaken with successive portions of acetic ether. The acetic ether solution was distilled to dryness, the residue taken up with water, the solution filtered and evaporated to dryness under reduced pressure. This residue was then dissolved in a mixture of alcohol and ether, and the solution filtered and evaporated. This method of purification was repeated several times. The tannin was finally treated with absolute ether and dried at 120° C. It yielded the following results upon combustion:

	Per Cent.	Oak Tannin.
Carbon	60·57	59·79
Hydrogen	5·38	5·08
Oxygen	34·05	35·13

Pinus Echinata.—The materials for these estimations were collected at St. David's, Pa.

Part.	Date.	Moisture.	Ash on Dry Basis.	Tannin on Dry Basis.	Remarks.
Whole bark	May 12, '94.	5·88	7·29	8·20	
" "	June 17.	8·41	3·42	6·11	
Inner "	July 27.	9·83	3·84	9·87	
Whole "	Aug. 28.	66·43	2·80	10·66	
" "	Oct. 3.	8·52	3·02	5·57	
" "	Nov. 4.	9·81	2·79	6·04	
" "	Dec. 12.	19·04	2·06	6·54	
" "	Jan. 20, '95.	8·92	2·98	7·905	
" "	Feb. 25.	7·22	2·03	7·70	Sap beginning to flow.

For the separation and purification of the tannin the following methods were applied: The dried bark was exhausted with acetone, the solvent recovered and the residue taken up in water and filtered. The clear solution was shaken with acetic ether, but this did not remove the tannin; the watery solution was saturated with salt and again shaken with acetic ether, but still the tannin was not removed. The saturated watery solution was then shaken with acetone, which only removed a part of the tannin. The acetone solution was

evaporated, the residue taken up in water, and this clear solution shaken with ether.

As this solvent did not remove anything, the watery solution was evaporated to dryness under reduced pressure; the residue dissolved in alcohol and ether, filtered and evaporated. This method of purification was repeated several times and finally gave the tannin in a puffed condition.

This tannin was completely soluble in water, and the watery solution was tested with the following reagents:

Reagent.	Pinus Echinata.	Quercus Robur.	Gallotannic Acid.
Copper sulphate and	No. ppt.	Ppt.	No. ppt.
Ammon. hydrate.	Purplish-brown ppt.	Red-brown ppt.	Brown ppt.
Pinewoodshavingand hydrochloric acid.	Violet color with so·· e green.	Violet color.	Slight green color.
Ferric chloride and	Green, turning yellowish brown and ppt.	Bluish-green color and green ppt.	Blue color and ppt.
Ammon hydrate.	Purple ppt.	Purple-brown ppt.	Purple ppt.
Ammonio-ferric sulphate.	Brownish-green color and ppt.	Bluish-green color and green ppt.	Blue color and ppt.

JUGLANDACEÆ.

Hicoria Laciniosa.—This sample of bark was collected at St. David's, Pa., July 12, 1894, and yielded on examination: Moisture, 9·65 per cent.; ash, on dry basis, 5·95 per cent.; tannin, on dry basis, 6·73 per cent.

Torotee.—This sample was sent to Professor Trimble from New Mexico, June 30, 1894, and yielded: Moisture, 9·56 per cent.; ash, on dry basis, 9·28 per cent.; tannin, on dry basis, 25·43 per cent.

Eucalyptus gum from Australia, July 16, 1894, yielded: Moisture, 17·53 per cent.; ash, on dry basis, ·12 per cent.; tannin, on dry basis, 20·61 per cent.

The purified tannin from this sample of gum was submitted to ultimate organic analysis with the following results, these being the average of three estimations: Carbon, 56·906 per cent.; hydrogen, 4·148 per cent.; oxygen, 38 946 per cent.

Notes on the acidity of the fruit of the sumachs, which is due to malic acid.

Species.	Moisture.	Acidity on Dry Basis.	Date of Collection.	Remarks.
Rhus copallina .	43·71	17·37	July 13, '94.	Blossom just before blooming.
" typhina .	26·68	13·43	" "	Berries well formed.
" "	11·17	13·12	Aug. 16.	" " "
" "	8·92	10·00	Sept. 16.	Leaves not completely turned in color.
" glabra . .	35·46	6·54	July 13.	Berries formed but not hairy.
" "	11·45	8·70	Aug. 16.	Berries fully developed.
" "	9·59	6·22	Sept. 16.	Leaves all turned.

In estimating the tannin, difficulty was experienced in percolating the infusion of the berries through the hide powder, the malic acid having the property to gelatinize the hide powder. It was found, by working with solutions of known strength of malic acid, that 1· gramme of hide powder absorbed ·0217 gramme of malic acid.

SOME CURRENT NOTES FOR FUTURE HISTORY.

By M. I. Wilbert.

According to published reports, pharmaceutic history is being made at a very rapid rate at the present time. This history, as recorded in the daily papers, does not always reflect creditably on the pharmaceutical profession. It must be admitted, however, that at times the reports as published are so perverted that they have lost all semblance to the true facts of the case.

Quite a humorous illustration of how news may be garbled was given by some of the Philadelphia papers a short time ago, when some of the members of a local association of retail druggists were discussing a plan to improve trade conditions; this was promptly reported as a scheme for forming a gigantic million-dollar retail drug store trust, with the avowed object to compel the sick and unfortunate to pay untold profits into the coffers of the prospective trust magnates.

Another flurry of rather a more serious nature was occasioned in New York City several months ago, when the daily papers devoted considerable space to reporting, and commenting on, the results of several series of investigations that had been made by the Board of Health of that city, with a view of inquiring into the probable

degree of adulteration or sophistication of drugs and medicinal preparations.

Unfortunately for all concerned, the officials of the Board of Health devoted their attention at first to investigating and reporting on a patented chemical, one that is not official in the United States Pharmacopœia, and one for which there are no generally known or easily applied tests to distinguish it from several other preparations of a similar nature.

The report of the initial investigations of the Board of Health had a tendency to discredit the motives that induced the inquiry; it has also had a tendency to vitiate the ultimate results that should have accrued.

A second series of investigations by the same Board, with the object of demonstrating the widespread use of wood alcohol in pharmacopœial preparations, in place of the official grain alcohol, disclosed a considerable amount of substitution, although it should be added that the use of wood alcohol appears to be confined entirely to preparations that were intended for external use. This aggressive action of the Board of Health of the City of New York appears to have stimulated other authoritative bodies in different parts of the country to make similar investigations. In many instances these investigations have occasioned more or less sensational reports to be made through the daily papers, though the majority of them have had only a local influence.

One unfortunate outcome of this particular crusade has been the passing of a bill by the Legislature of the State of New York, making it a criminal offence for a druggist to substitute any preparation, other than the one intended by the physician, on any prescription or order.

Fortunately, the wording of this bill was so ambiguous, and its provisions so difficult of execution, that the Governor of the State refused to affix his signature to the same.

This is, of course, only one of a number of similar measures that will be introduced into the several State Legislatures in the near future. A recent editorial in the *American Druggist* (May 11, 1903), in commenting on this mischievous tendency of modern legislative bodies says: "One of the most distressing evils of our own time is the blundering and bungling legislator, the meddling and muddling law-maker who seeks to remedy real or imaginary social or political

faults by measures inspired by his own ignorance and prejudice, or by the cupidity of persons who might be benefited by such laws." Unfortunately, the above statement is too true, and as a consequence we are suffering from an oversupply of what might be called restrictive legislation. On the other hand, measures that are progressive in their tendency, and would result in ultimate good for the community at large, are entirely ignored. As an illustration of this we may cite the bill that was introduced into the Pennsylvania State Legislature, with the object of making it compulsory for candidates for examination as registered pharmacists to be graduates of a recognized school of pharmacy.

The Cocaine Habit.—The reported excessive use of cocaine has attracted the attention of law-makers in a number of States. Several of the State Legislatures have already adopted bills restricting the legitimate sale of this chemical, while in others similar measures are pending.

The Governor of Pennsylvania has recently approved a bill that had been passed by the State Legislature, which provides: "That no person shall sell, furnish, or give away cocaine, or any patent or proprietary remedy containing cocaine, except on the written prescription of a registered physician, or of a dentist, or of a veterinarian; nor shall any such prescription be refilled; nor shall any physician, dentist or veterinarian prescribe cocaine or any patent or proprietary remedy containing cocaine, for any person known to such physician, dentist or veterinarian to be an habitual user of cocaine."

"Provided that provisions of this act shall not apply to persons engaged in the wholesale drug trade, regularly selling cocaine to persons engaged in the retail drug trade."

Persons violating any of the provisions of this act are to be sentenced to pay a fine of not more than $100, or to undergo an imprisonment of not more than six months, or both, at the discretion of the court.

The American Medical Association, at its fifty-fourth annual session held at New Orleans, May 5, 6, 7 and 8, 1903, transacted considerable business that is of more than passing interest to the pharmacist. Among other matters, the "Principles of Medical Ethics" that were endorsed at this session contain several sections that more or less directly involve the members of the pharmaceutical profession. The first of these is section 8, of article 1, chapter 2:

"It is equally derogatory to professional character for physicians to hold patents for any surgical instruments or medicines; to accept rebates on prescriptions or surgical appliances; to assist unqualified persons to evade the legal restrictions governing the practice of medicine; or to dispense or promote the use of secret medicines; for if such nostrums are of real efficacy any concealment regarding them is inconsistent with beneficence and professional liberality, and if mystery alone give them public notoriety, such craft implies either disgraceful ignorance or fraudulent avarice. It is highly reprehensible for physicians to give certificates attesting the efficacy of secret medicines or other substances used therapeutically."

The other item is section 5 of chapter 3:

"It is the duty of physicians to recognize and by legitimate patronage to promote the profession of pharmacy, on the skill and proficiency of which depends the reliability of remedies; but any pharmacist who, although educated in his own profession, is not a qualified physician, and who assumes to prescribe for the sick, ought not to receive such countenance and support. Any druggist or pharmacist who dispenses deteriorated or sophisticated drugs, or who substitutes one remedy for another designated in a prescription, ought thereby to forfeit the recognition and influence of physicians."

To these two sections every fair-minded pharmacist will cheerfully subscribe; for, if lived up to on the part of the several members of the two professions, they would go far to eliminate many of the existing abuses, and also have a marked tendency of furthering and facilitating the demonstration of scientific truths, without which no real progress can be made.

One other action of the American Medical Association that may contribute materially to promote the progress of medical science is the proposed admission of pharmacists as members of the section on Materia Medica, Pharmacy and Therapeutics.

The original amendment, as offered to the House of Delegates by the Business Committee, was for Pharmaceutic Associate Members, and read as follows: "Pharmacists who are recognized graduates of pharmaceutic schools or colleges and are members of the American Pharmaceutical Association may be admitted as associate members on recommendation of the officers of the section on Materia Medica, Pharmacy and Therapeutics, subject to approval by a majority of the members of the section."

This amendment was subsequently referred back to the committee, and at a later meeting of the House of Delegates the following substitute was adopted:

"Reputable pharmacists may be admitted as pharmaceutic members on recommendation of the officers of the section on Materia Medica, Pharmacy and Therapeutics, subject to approval by a majority vote of the members of the section, the names of such members to be sent to the secretary by the secretary of the section."

The provisions for dental members of the section on Stomatology, adopted at the same time, require that the candidate have the degree of D.D.S. from some recognized school of dentistry, and also be a member in good standing of some recognized dental society.

The section on Materia Medica, Pharmacy and Therapeutics also adopted several resolutions that are of more or less interest to pharmacists. One of these, relating to the abuse of patent medicines, has one section that refers to medicines used by physicians. This is section 5 of the resolution, and reads: "That manufacturers be requested to print the scientific or chemical name under the trade name of all pharmaceutic or chemical preparations."

In another set of resolutions, in which the metric system of weights and measures is endorsed, the section recommends the proposed international standard dropper and also the use of 5 c.c. as the equivalent of 1 teaspoonful and of 15 c.c. as the equivalent of 1 tablespoonful.

REVIEWS AND BIBLIOGRAPHICAL NOTICES.

THE INTERNAL SECRETIONS AND THE PRINCIPLES OF MEDICINE. By Charles E. de M. Sajous, M.D. Volume I, with forty-two illustrations. Philadelphia: F. A. Davis Company. 1903.

The investigator busily occupied, as he usually is, in a more or less limited domain, is likely to lose sight of the relation of his work to that of his colaborers in other directions in the building up of fundamental principles of science. Fortunately, there always have been some men who have watched the progress of the various investigators and co-ordinated these results into a system of principles. This work becomes more and more difficult as the number of investigators increases and the field of operation widens. Dr. Sajous has been since 1888 closely following the development of the various branches of medical science, correlating the facts of investigators,

which he has published in the "Annual of the Universal Medical Sciences," and in the "Analytical Cyclopedia of Practical Medicine." The results of these years of labor have given Dr. Sajous an insight into what may be considered the essential elements in the construction of a more rational system of medicine, and while we may not be prepared to accept or reject Dr. Sajous' conclusions, his ability as a thinker and student entitle his views to the respect and consideration of men of science. To say the least, his theories are ingenious.

The secretion of the adrenals was traced as far as the pulmonary alveoli, but not beyond. Here it was found to hold in combination the various constituents of hæmoglobin, and to endow both the latter and the plasma with their affinity for oxygen. Prevailing views as to the chemistry of respiration were thus radically transformed, and our knowledge of the manner in which the blood-pigments were held together, likewise. This portion of the inquiry also revealed that, while hæmoglobin absorbed its share of adrenal secretion and oxygen, the plasma did likewise. It thus became evident that the red corpuscles were not only carriers of oxygen, and that the blood-plasma played an important part in the distribution of this gas. Indeed, he subsequently ascertained that the red corpuscles were secondary factors in this important function, *i.e.*, mere carriers, pack-mules, as it were, and that it was the oxygen-laden adrenal secretion dissolved in the plasma itself which carried on all the oxidation processes of the organism.

The many physiological problems awaiting solution appear in quite a new light. The ease with which the oxygen carried by the plasma could penetrate the minute vascular net-works of all cellular elements not only furnished a clue to the physiological chemistry of the latter, but it also led to the discovery that various structures, the functions of which were unknown, were in reality blood-channels, or rather plasma-channels.

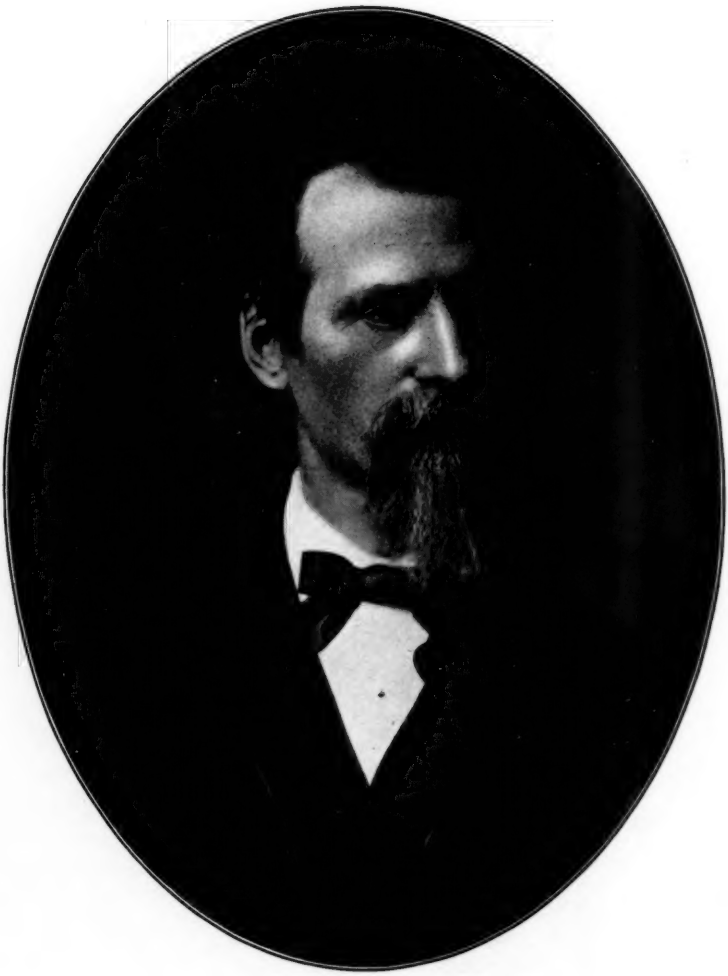
The functions of the other ductless glands were studied. Investigations then showed that the adrenals were directly connected with the *anterior pituitary body*, and that this diminutive organ, hardly as large as a pea, and now thought to be practically functionless, proved to be the most important organ of the body, as governing centre of the adrenals, and, therefore, of all oxidation processes. In general diseases what has been termed the patient's "vitality," or "vital resistance," thus became ascribable to fluctuations in the

anterior pituitary body's functional efficiency. The functional efficiency of this organ was found to be maintained through the *thyroid gland.* The thyroid gland, the anterior pituitary, and the adrenals were found to be functionally united: *i. e.*, to form an autonomous system, which he terms the "adrenal system."

Radical changes in prevailing doctrines as to the manner in which general infections, or other forms of poisoning, produced their effects on the organism thus seemed to impose themselves, and led to the conclusion that what was now considered as symptoms of infection or poisoning are all manifestations, more or less severe, of *overactivity or insufficiency of the adrenal system.* Indeed, *the physiological action of remedies was also traced to the anterior pituitary body*, the governing centre of this system.

The bearing of this discovery upon the prevailing interpretation of the pathogenesis and treatment of disease is well shown by the manner in which it at once elucidated our knowledge of even the greater scourges of humanity. The symptomatology of Asiatic cholera, for example, was found to be a counterpart of the symptom-complex of advanced adrenal insufficiency, and due to the effects of cholera-toxins upon the anterior pituitary body. The only treatment of any value whatever, as is well known, is early and active stimulation: *i.e.*, the use of agents which, as does the thyroid's active principle, reawaken the functional activity of this organ. Cholera infantum, arsenic poisoning, various toxalbumins, and other intoxications produce identical symptoms; all these proved likewise to be syndromes due primarily to adrenal insufficiency.

Dr. Sajous seems to us to have shown that the adrenal system is the source of the secretion which, with the oxygen of the air, forms the oxidizing substance of the blood-plasma. It has also revealed the origin and mode of distribution of the bodies with which this oxygen directly or indirectly combines; *i.e.*, peptones, myosinogen, fibrinogen, hæmoglobin, and myelin, to insure the continuation of life and the efficiency of all organic functions. Finally, it has suggested that in addition to these agencies, all leucocytes and, under certain circumstances, the plasma, contain a protective agency, trypsin, which, with Metchnikoff's phagocytic cells, serves to destroy micro-organisms and convert their toxins and other albuminoid poisons into harmless products. Considered jointly, these various factors seem to represent the aggregate of vital phenomena.



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From a photograph by Gutekunst.